## Report as of FY2009 for 2008VT36B: "Improvement of Phosphorus Load Estimates through the use of Enzyme-Hydrolysis Measures of Phosphorus Bioavailability"

## **Publications**

- Articles in Refereed Scientific Journals:
  - ◆ Johnson, N.R. and Hill, J.E. 2010 (accepted) Phosphorus composition of a poultry manure-amended soil via enzymatic hydrolysis: demonstration of a high-throughput method and hints on enzyme-labile P Soil Science Society of America Journal
  - ♦ Giles, C.D., Cade-Menun, B., and Hill, J.E. (under review) The Inositol Phosphates in Soils and Manures: Abundance, Cycling, and Measurement.
- Other Publications:
  - ♦ Johnson, Nicholas R., and J.E. Hill, August 2009. Phosphorus composition of wet and dried poultry litter-amended soil by enzyme hydrolysis and solution 31P-NMR. 238th ACS National Meeting Presentation.
  - ♦ Hill, J.E., 2009, Phytate: Movement and Transformation in the Landscape, Great Lakes Phosphorus Forum, Poster, July.
  - ♦ Giles, Courtney D., Barbara Cade-Menun, and J.E. Hill, 2009, Phosphorus mobility and transformation in a poultry manure-amended soil tracked over time and depth, Presentation, August.
  - ◆ Johnson, Nicholas, Barbara Cade-Menun, and J.E. Hill, 2009, Hydrolysis as a Tool for Measuring Bioavailable P in Dairy Manure Storage and Treatment Systems, Presentation, November.

## **Report Follows**

Title: Improvement of Phosphorus Load Estimates through the use of Enzyme-Hydrolysis

Measures of Phosphorus Bioavailability

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## Notable Accomplishments Narrative

Microorganisms alter the forms of phosphorus in soils and sediments over time. Some forms, such as orthophosphate, are more available to cyanobacteria and crop plants. We need to be able to measure such bioavailability in order to improve crop soil fertility as well as decrease the phosphorus in runoff from agricultural fields. Our present soil analysis methods for phosphorus forms are either very expensive (e.g. <sup>31</sup>P-NMR) or do not reveal the total amount of bioavailable phosphorus in the sample (e.g. Modified Morgan P). This lack of knowledge hinders our ability to manage our agricultural soils and thus the watershed. In the past decade, pioneering research conducted at the USDA Plant, Soil and Water Laboratory in Orono, Maine studying animal manures has lead to the development of a method for analyzing forms of bioavailable phosphorus using enzymes. The first objective of this study is to modify this enzymatic method so that it can be applied to characterize Vermont soil systems. The second objective of this study is to employ the modified enzymatic method to Vermont soil systems primarily from the Lake Champlain Watershed area, where we are most concerned about phosphorus pollution entering the Lake.

We have successfully modified and employed a robust, efficient and sensitive phosphohydrolase based assay of soil P forms on a time series of manure-amended Vermont soil samples. We assessed the substrate specificity of several commercially available phosphohydrolases against 13 P compounds commonly found in soil. Knowledge of this specificity allowed us to select a cocktail of enzymes that facilitated the classification of soil P in into three categories: inorganic P, enzyme labile simple monoester P + phytate-like P, and enzyme labile nucleic acid P. Furthermore, we improved the robustness and efficiency of this assay by adapting it to a microplate reader format which allowed us to process a large number of samples quickly. Using this adapted tool we looked at a poultry manure-amended Vermont soil over a period of 10 weeks. We were able to both track three P forms over time and confirm observed proportions of P with <sup>31</sup>P-NMR spectroscopy. This has resulted in an accepted publication to the Soil Science Society of America Journal and five presentations at national meetings (Great Lakes Phosphorus Forum, ASA-SSSA-CSSA, and ACS).

We have also applied the method to measure the potential for Vermont soils to release orthophosphate under saturated and anoxic conditions. The data for this work has been collected and is presently being assessed for presentation and publication in a peer-reviewed journal. Knowledge gleaned from this study will affirm the usefulness of this assay to track enzyme labile P forms and provide a foundation for future work identifying specific mechanisms facilitating the release and microbial modification of labile soil P. Students supported by this project include a Ph.D. candidate, an MS candidate, and an undergraduate who recently transitioned to the MS program. All students are part of the Civil and Environmental Engineering Program at the University of Vermont.